



Figure 1

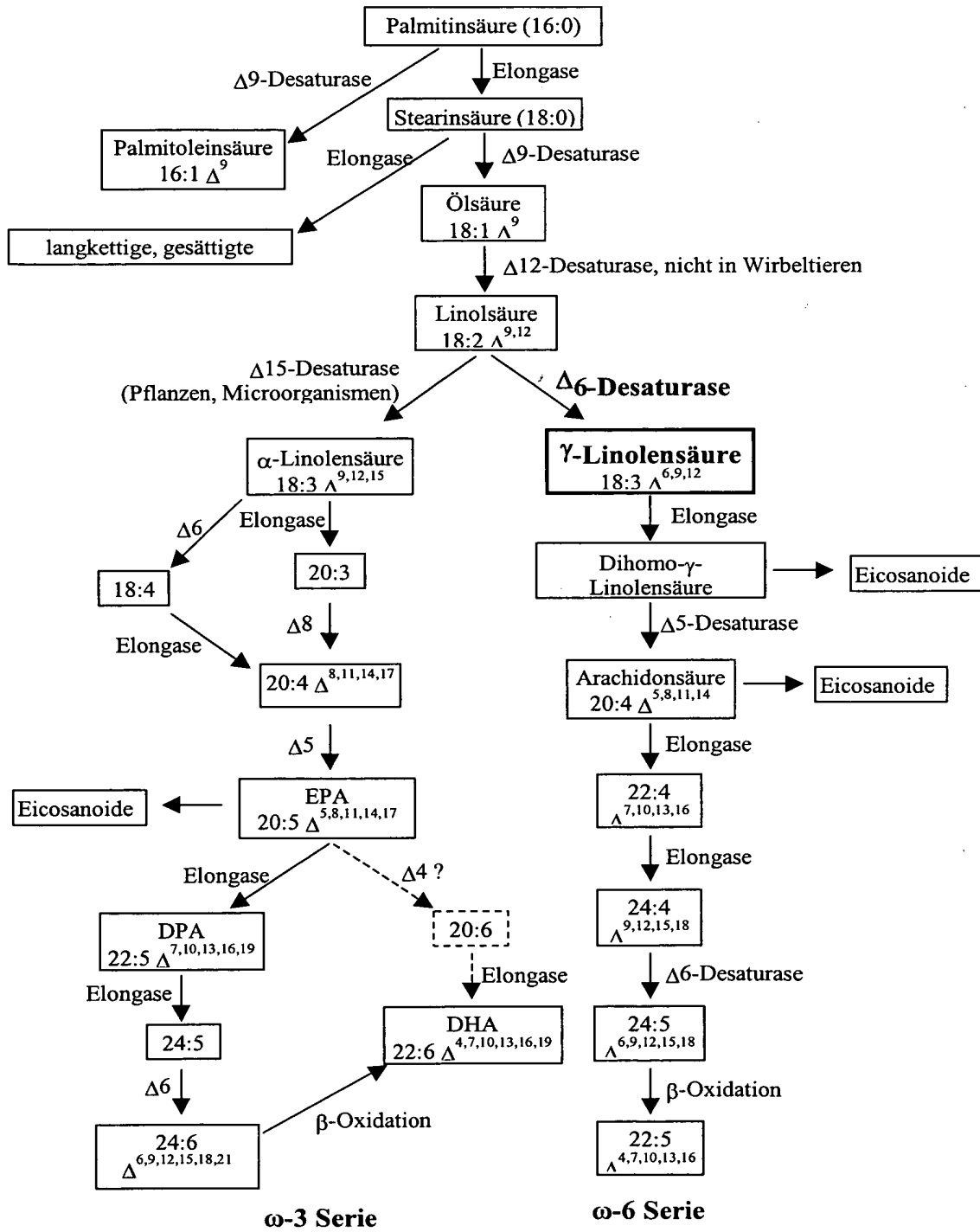




Figure 2

BLASTP 2.0.8 [Jan-05-1999]

Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

Query= T.thermophila, delta-6-Desaturase (352 letters)

Database: /LION/data/db/fast/nrdb

387,705 sequences; 119,829,732 total letters

Sequences producing significant alignments:	Score (bits)	E Value
trembl AF078796 AF078796_1 gene: "des-5"; product: "delta 5 fat...	79	4e-14
trembl AF031477 AF031477_1 product: "delta6-fatty-acid-desatura...	79	4e-14
trembl Z81122 CET13F2_1 gene: "T13F2.1"; Caenorhabditis elegan...	78	1e-13
trembl Z70271 CEW08D2_2 gene: "W08D2.4"; Caenorhabditis elegan...	78	1e-13
trembl AF005096 AF005096_1 product: "desaturase/cytochrome b5 p...	70	3e-11
trembl AJ222980 PPAJ2980_1 gene: "des6"; product: "delta6-acyl-...	69	6e-11
trembl U79010 BOU79010_1 product: "delta 6 desaturase"; Borago...	67	2e-10
trembl AC005397 AC005397_14 gene: "T3F17.14"; product: "putativ...	67	2e-10
trembl AF007561 AF007561_1 product: "delta-6-Desaturase"; Bora...	66	4e-10
tremblnew AF126799 AF126799_1 product: "delta-6 fatty acid desa...	64	2e-09
tremblnew AF126798 AF126798_1 product: "delta-6 fatty acid desa...	63	3e-09
trembl AF031194 AF031194_1 gene: "S276"; product: "S276"; Trit...	62	6e-09
tremblnew AB021980 AB021980_1 product: "delta-6 fatty acid desa...	62	6e-09
tremblnew AL078610 SCH35_12 gene: "SCH35.42c"; product: "putati...	62	8e-09
trembl AJ224160 BNAJ4160_1 gene: "sld1"; product: "delta-8 sphi...	60	2e-08
trembl AC004770 AC004770_2 product: "BC269730_2"; Homo sapiens...	60	3e-08
trembl AJ224161 ATAJ4161_1 gene: "sld1"; product: "delta-8 sphi...	59	6e-08
tremblnew AL050118 HSM800210_1 gene: "DKFZp586C201"; product: "...	57	2e-07
trembl AB022097 AB022097_1 product: "delta 5 fatty acid desatur...	57	2e-07
trembl X87143 HACYTB5RN_1 product: "cytochrome b5 containing fu...	50	2e-05
trembl Y08460 MMMD5_1 gene: "Mdes"; product: "Mdes protein"; ...	50	3e-05
trembl AF001394 AF001394_1 product: "fatty acid desaturase/cyto...	46	4e-04
trembl AF002668 HSAF2668_1 product: "MLD"; Homo sapiens putati...	46	5e-04
swiss Q08871 LLCD_SYNY3 LINOLEOYL-COA DESATURASE (EC 1.14.99.25...	43	0.003

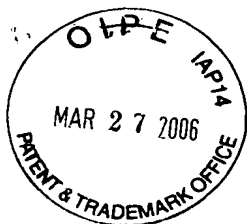


Figure 3A

>aageneseq|W95504|W95504 Mortierella alpina delta 6 desaturase. Length = 457

Score = 89.7 bits (219), Expect = 4e-18

Identities = 102/422 (24%), Positives = 152/422 (35%), Gaps = 88/422 (20%)

Query: 9 EIVLENKPELLNEYKFIYKDTEYDCTEYAKSNKHPGGLNFLNLFIDEKQDLTEYFRTLHS 68
E + E K + + I + YD E+ HPGG L +D T+ F T H
Sbjct: 19 EALNEGKKDAEAPFLMIIDNKVYDVREFVPD--HPGGSVILT--HVSKDGTDFVDFTFHP 73

Query: 69 KQALKILKSFPKTKGAKQEETE-SSKRFSILKKLKLHLEPNWPIEIG---LFLTTFTLF 123
+ A + L +F + + + + F+ +KL+ LF+ + F +F L
Sbjct: 74 EAAWETLANFYVGDIDESDRDIKNDDFAAEVRKLRTLFQSLGYDSSKAYYAFKVSFNLC 133

Query: 124 VTGCLT---QKW-----YFSIPLLVLMQIISGWIGHSMNHNRPILR----KFALVY 168
+ G T KW S LL L GW+ H H++ R F
Sbjct: 134 IWGLSTVIVAKWGQTSTLANVLSAALLGLFWQQCGWLAHDFLHHQVFQDRFWGDLFGAFL 193

Query: 169 APLCGGFSNKKWGRKHNQHMHFTNNILKDEDIQ-HDYKLWQ----- 208
+C GFS+ WW KHN HH N +D DI H W
Sbjct: 194 GGVCQGFSSSWWKDKHNTHHAAPNVHGEDPDIDTHPLLTWSEHALEMFS DVPDEELTRMW 253

Query: 209 -----FP---FLFLKWKLDSIL-----ASYEFEGIFLALHWV 238
FP F L W L SIL E + LA+HW
Sbjct: 254 SRFMVLNQTFYFPILSFARLSWCLQSILFVLPNGQAHKPSGARVPISLVEQLSLAMHWT 313

Query: 239 LLFNQNFYIV-----ILSELIAGFFSASILVGNHEN--EMKFERRITLPFFEHI 286
F + ++S+ + G A + NH + E + + FF QI
Sbjct: 314 WYLATMFLFIKDPVNMLVYFLVSQAVCGNLLAIVFSLNHNGMPVISKEEAVDMDFFTKQI 373

Query: 287 AASRNYAFHDIFSLLIMGGMQYQTEHHFFPQIPFYRLPKARVIIAEELKKWNLKIHEGPI 346
R+ +F+ GG+ YQ EHH FP +P + K + + KK+N++ H +
Sbjct: 374 ITGRD-VHPGLFANWFTGGLNYQIEHHLFSPMPRHNFSKIQPAVETLCKKYNVRYHTTGM 432

Query: 347 FE 348
E
Sbjct: 433 IE 434



Figure 3B

>trembl|AF031477|AF031477_1 product: "delta6-fatty-acid-desaturase";
Caenorhabditis elegans delta6-fatty-acid-desaturase mRNA, complete cds.
//:gp|AF031477|3088520 product: "delta6-fatty-acid-desaturase";
Caenorhabditis elegans delta6-fatty-acid-desaturase mRNA, complete cds.
Length = 443

Score = 79.2 bits (192), Expect = 4e-14

Identities = 100/390 (25%), Positives = 132/390 (33%), Gaps = 95/390 (24%)

Query: 41 KHPGGLNFLNLFIDEKQDLTEYFRTLH--SKQALKILKSFPKTGAKQE--ETESSKR--- 93
KHPGG D T F H S QA K L K G E E + KR
Sbjct: 28 KHPGGAVIEQY---RNSDATHIFHAFHEGSSQAYKQLDLLKKHGEHDEFLEKQLEKRLDK 84

Query: 94 -----FSILKKKLKH--LFEPNWPIEIGLFLTTFTLFVTGCLTQ 130
F L++KL L + N + ++T ++ Q
Sbjct: 85 VDINVSAYDVSVAQEKKMVESFEKLRQKLHDDGLMKANETYFLFKAISTLSIMAFAYLQ 144

Query: 131 K--WYF-SIPLLVLQMIIISGWIGHSMNHNHNR----NPILRKFALVYAPLCGGFSNKWWGRK 183
WY S LL L GW+ H H + P+ +L + GFS WW K
Sbjct: 145 YLGWYITSACLLALAWQQFGWLTHEFCHQOPTKNRPLNDTISLFFGNFLQGFSRDWWKDK 204

Query: 184 HNQHMMFTNNILKDEDI-----QHDYKLWQFPFLF 213
HN HH TN I D DI QH Y P L
Sbjct: 205 HNTTHAATNVIDHDGDIDLAPLFAFIPGDLCKYKASFEKAILKIVPYQHLYFTAMLPLMR 264

Query: 214 LKWKLDASILASYE-----FEGIFLALHWVLLFNQNFYI-----VILS 251
W S+ + E +E + HW +F Q F + I+S
Sbjct: 265 FSWTGQSVQWVFKENQMEYKVYQRNAFWEQATIVGHAWVVFYQLFLLPTWPLRVAYFIIS 324

Query: 252 ELIAGFFSASILVGNHENEMKF--ERRITLPFFEHQIAASRNYAFHDIFSLIMGGMQYQ 309
++ G A ++ NH + K+ RI F QI +RN L GG+ YQ
Sbjct: 325 QMGGGLLIAHVVTFNHNSVDKYPANSRILNNFAALQILTTRNMTSPSPFIDWL-WGGLNLYQ 383

Query: 310 TEHHFFPQIPFYRLPKARVIAEELKKWNL 339
EHH FP +P L + E K+ NL
Sbjct: 384 IEHHLFPTMPRCNLNACVKYVKEWCKENNL 413



Figure 3C

>trembl|U79010|BOU79010_1 product: "delta 6 desaturase"; Borago officinalis delta 6 desaturase mRNA, complete cds. //:gp|U79010|2062403 product: "delta 6 desaturase"; Borago officinalis delta 6 desaturase mRNA, complete cds. Length = 448

Score = 67.1 bits (161), Expect = 2e-10
Identities = 100/414 (24%), Positives = 154/414 (37%), Gaps = 100/414 (24%)

```
Query: 6   TQEEIVLENKPELLNEYKFIYKDTEYDCTEYAKSNKHPGGLNFLNLFIDEKQDLTEYFRT 65
          T +E+  +KP  +      +   YD +++ K   HPGG  L      Q++T+ F
Sbjct: 10  TSDELKNHDKP---GDLWISIQGKAYDVSDWVKD--HPGGSFPLKSLAG--QEVTDAFVA 62

Query: 66  LHSKQALKILKSFPKGTAKQEE---TESSK-----RFSILKKKLLKHLFEPNWPIE 112
          H      K L  F  TG  ++   +E SK              +  +  KK  +F      I
Sbjct: 63  FHPASTWKNLDKF-FTGYYLKDYSVSEVSKDYRKLVFEFSSKMGLYDKKGHIMFATLCFIA 121

Query: 113 IGLFLTTF-TLFVTGCLTQKWYFSIPLLVLQMIIISGWIGHSMNHN---NPILRKFWLVY 168
          +  ++ +  LF  G L      FS  L+  + I SGWIGH  H      +  L KF  ++
Sbjct: 122 MLFAMSVYGVLFCEGVLVH--LFSGCLMGFLWIQSGWIGHDAGHYMVVSDSRLNKFMGIF 179

Query: 169 APLC-GGFSNKWWGRKHNQHMHFTNNILKDEDIQH----- 202
          A  C  G S  WW  HN HH+  N++  D D+Q+
Sbjct: 180 AANCLSGISIGWWKWNHNAHHIACNSLEYDPLQYIPFLVSSKFFGSLTSHFYEKRLTF 239

Query: 203 -----DYKLWQFPFLFLKWKLDSILASY-----YEFEGIFLALHWVLL- 240
          Y+ W F  +      +L+  + S              +E G  +  W  L
Sbjct: 240 DSLSRFFVSYQHWTFYPIMCAARLNMYVQSLIMLLTKRNVSYRAHELLGCLVFSIWYPLL 299

Query: 241 -----FNQNFYIVILSELIAGF-----FSASILVGNHENEMKFERRITLPFFEHQ 285
          +  +      VI S  + G              FS+S+ VG  +      FE++ T      +
Sbjct: 300 VSCLPNWGERIMFVIASLSVTGMQQVQFSLNHFSSSVYVGKPKGNNWFEKQ-TDGTLDIS 358

Query: 286 IAASRNYAFHDIFSLLIMGGMQYQTEHHFFPQIPFYRLPKARVIIAEELKKWNL 339
          ++ FH              GG+Q+Q EHH FP++P  L K      + E  KK NL
Sbjct: 359 CPPWMDW-FH-----GGLQFQIEHHLFPKMPCNLRKISPYVIELCKKHNL 403
```



Figure 3D

>tremblnew|AF126799|AF126799_1 product: "delta-6 fatty acid desaturase";
Homo sapiens delta-6 fatty acid desaturase mRNA, complete cds.
//:gp|AF126799|4406528 product: "delta-6 fatty acid desaturase"; Homo
sapiens delta-6 fatty acid desaturase mRNA, complete cds. Length = 444

Score = 63.6 bits (152), Expect = 2e-09

Identities = 92/390 (23%), Positives = 152/390 (38%), Gaps = 88/390 (22%)

Query: 31 YDCTEYAKSNKHPGGLNFLNLFIDEKQDLTEYFRTLHKSQAL--KILK-----SFPKTGA 83
Y+ T++ S +HPGG + + E D T+ FR H K LK
Sbjct: 44 YNITKW--SIQHPPGQRVIGHYAGE--DATDAFRAFHPDLEFVGKFLKPLLIGELAPEEP 99

Query: 84 KQEETESSK--RFSILKKKLLK--HLFEPNWPPIEIGLF-----LTTFTLFVTGCLTQ 130
Q+ ++SK F L+K + +LF+ N + L + FT+F G
Sbjct: 100 SQDHGKNSKITEDFRALRKTAEDMNLFKTNHVFFLLLLAHIIALESIAWFTVFYFGNGWI 159

Query: 131 KWYFSIPLLVLMQIISGWIGHSMNH-----NRNPILRKFFALVYAPLCGGFSNKWWGRK 183
+ +L Q +GW+ H H N ++ KF + + G S WW +
Sbjct: 160 PTLITAFVLATSQAQAGWLQHDYGHLSVYRKPKWNHLVHKFVIGHLK--GASANWWNHR 216

Query: 184 HNQHMMFTNNILKDEDIQ--HDYKL--WQFPFLFLKWKL-----DSIL 222
H QHH N KD D+ H + L WQ P + K KL ++
Sbjct: 217 HFQHHAKPNIFHKDPDVNMLHVFLGEWQ-PIEYGGKKLKYLPYNHQHEYFFLIGPPLLI 275

Query: 223 ASYYEFEGI-----FLALHWVLLFNQNFYIV-----ILSELIAGFFSASILVGNH- 267
Y++++ I ++ L W + + F+I IL L+ F + + +H
Sbjct: 276 PMYFQYQIIMTMIVHKNWVDLAWAVSYIIRFFITYIPFYGILGALL--FLNFIRFLESHW 333

Query: 268 -----ENEMKFERRITLPFFFEHQIAASRYA---FHDIFSLIMGGMQYQTEHHFFP 316
M+ ++ +F Q+ A+ N F+D FS G + +Q EHH FP
Sbjct: 334 FVWVTQMNHIVMEIDQEAYRDWFSSQLTATCNVEQSFFNDWFS----GHLNFQIEHHLFP 389

Query: 317 QIPFYRLPKARVIIAEELKKWNLKIHEGPI 346
+P + L K ++ K ++ E P+
Sbjct: 390 TMPRHNHLHKIAPLVKSLCAKHGIEYQEKPL 419



Figure 3E

>swiss|Q08871|LLCD_SYNY3 LINOLEOYL-COA DESATURASE (EC 1.14.99.25)
(DELTA(6)-DESATURASE).//:trembl|L11421|SSD6DS_1 product: "delta-6-Desaturase"; Synechocystis sp. delta-6-Desaturase gene, complete cds.
//:trembl|D90914|SSD914_112 gene: "des6"; product: "delta-6-Desaturase";
Synechocystis sp. PCC6803 complete genome, 16/27, 1991550-2137258.
//:pironly|S35157|S35157 Delta(6)-desaturase - Synechocystis
sp.//:gp|D90914|1653589 gene: "des6"; product: "delta-6-Desaturase";
Synechocystis sp. PCC6803 complete genome, 16/27, 1991550-2137258.
//:gp|L11421|349563 product: "delta-6-Desaturase"; Synechocystis sp. delta-6-Desaturase gene, complete cds. Length = 359

Score = 43.4 bits (100), Expect = 0.003

Identities = 63/288 (21%), Positives = 101/288 (34%), Gaps = 61/288 (21%)

Query: 120 FTLFVTGCLTQKWYFSIPLLVLQMIIISGWIGHSMNHN---NP-ILRKFAVYAPLCGGF 175
F LF + + L + + S +GH NHN NP I R + Y + G
Sbjct: 57 FVLFAFVIFPVRLGCMVLAIALAAFSFNVGHDANHNAYSSNPHINRVLGMTYDFV--GL 114

Query: 176 SNKWWGRKHNO-HHMFTNNILKDEDIQHDYKLWQFPFL-----FLKWKLDLSILAS 224
S+ W +HN HH +TN + D +I D + P F W L +
Sbjct: 115 SSFLWRYYRHNHLYHTYTNILGHDVEIHGDGAVRMSPEQEHVGIYRFQQFYIWGLYLFIPF 174

Query: 225 YYEFEGIFLAL-----HWVLLFNQNFYIVILS-----ELIAGFFSASILVG 265
Y+ ++L L H + F +L L GF +L+G
Sbjct: 175 YWFLYDVYLVNLNKGKYHDHKIPPFQPLELASLLGIKLLWLGYVFGFLPLALGFSIPEVLIG 234

Query: 266 NHENEMKFERRI-TLPFFE-----QIAASRNYAFHDIFSL 300
M + + T+ H QI + N+A ++ F
Sbjct: 235 ASVTYMTYGIVVCTIFMLAHVLESTEFLLTPDGESGAIDDEWAICQIRTTANFATNNPFWN 294

Query: 301 LIMGGMQYQTEHHFFPQIPFYRLPKARVIIAEELKKWNLKIHEGPIFE 348
GG+ +Q HH FP I P+ II + +++ ++ P F+
Sbjct: 295 WFCGGLNHQVTHHLFPNICHIIHYPQLENIIDKVCQEFQVEYKVYPTFK 342

Figure 4

M.alpina	1	MAAAPSVRTTFAEVLNAEALNECKKDAEAPFLMIIDNKKVYDVREFVP--DHPGG
C.elegans	1	MVLREQE---HEPFFIKIDGKWCQIDDAVLR-SHPGG
B.officinalis	1	MAAQIKKYITSDLELKNHDK---PGDLWISIQGKAYDVSDWVK--DHPGG
M.musculus	1	MKGKGNQGEGSTERQAPMPTFRWEEIQKHNLRTDRWLVIDRKVYNNVTKWSQ--RHPGG
T.thermophila	1	MGVDKTQEEIVLENKPELLNEYKFIYKDTXDCTEYAKSNKHPGG
M.alpina	54	SVILTHVCK--DGTDFDTFHPEA---AWETLANFYVGDIDESDRDIKND---D--
C.elegans	34	SAITTYKNM--DATTVFHTHTGSKEAYQWLTELKKECPTQEPPIPDIKDDPIKGIDDV
B.officinalis	45	SFPLKSLAQ--EVTDAFVAFHPAS---TWKNLDKFFTGYLKDYSVSEVS-----
M.musculus	57	HRVIGHYSCE--DATDAFRAFHLDLD--FVGKFLKPLLIIGELAPEEPSLDRG-----
T.thermophila	46	LNFLNLFIDEKQDLTEYFRTHLSKQ---ALKILKSFPKTGAQKEE--TESS-----
M.alpina	100	FAAEVR-----KLRTLFSQSLGYDSSKAYYAFKVSFNLCIWGLSTVIVAKW
C.elegans	91	NMGTFNISEKRSQINKSFTDLRMVRVRAEGLMDGSPFYIRKI-----LETIFTILFAFY
B.officinalis	91	KDYR-----KLVEFSKMGLYD-KKGHIIMFAT-LCFIAMLFAMSVYGV
M.musculus	105	KSSQITEDFR-----ALKKTAEDMNLKTNHLEFFLLLSHIIVMESLAWFILSYF
T.thermophila	92	KR-----FSILKKKLKHLFEPNWPPIEI-----GLFLTFTTLLFVT
M.alpina	146	GQTSTLANVLSAALLGLFWQQCGWLAHDFLHHQVFQDRFWGDLFGAFILGGVCQ-----
C.elegans	146	LQYHTYY-LPSAILMGVAVQQQLGWLIIHEFAHHQLFKNRYNDLASYFVGNFLQVSHIFNN
B.officinalis	133	CEGVLVHLFSGCLMGFLWIQSGWIGHDACHYMWVSDSRLNKFMCIFAANCL-----
M.musculus	155	GTGWIPT-LVTAFVLA TSQAQAGWLQHDYGHLSVYKKS IWNHVHVKFVIGHLK-----
T.thermophila	126	GCLTQKW-YFSIPLLVLMQIIISGWIGHSMNHNRPILR-----KFALVYAPLCG-----

199 M.alpina CFSSSWKDKHNTTHAAPNVHGEDPDIDTHPLLTWSEHALEMFS--VPDEELTRMWSRF
 205 C.elegans GFSSGGWKEQHNVHHAATNVVGRDGLDLVPPFYATVAEHLNNYS---QDS---WVMT
 186 B.officinalis GISIGWKNHNAHHIAACNSLEYDPDLQYIPFLVSSKFFGSLTSHFYEKRLTFDLSLRF
 207 M.musculus GASANWNHRHFQHHAKPNIIFHKDPDIKSLHVFLGEWQPLEYG---KKK---LKYL
 174 T.thermophila GFSNKKWGRKHNQHHMFTNNILKDED-----I-----

257 M.alpina MVLNQTWFEYFPILSFARLSWCLQSILFVLPNGQAHKPSGARVPISLVEQLSLAMHWTWYL
 256 C.elegans LFRWQHWHWTFMIPFLRLSWLLQSIIFVSQMP-THYYDYRNP-TAIYEQVGLSLHWAWSL
 246 B.officinalis FVSYQHWTFFPIMCAARINMYVQSLIMILTKR---NVSYRA---QELLGCLVFSIWYP
 258 M.musculus PYNHQHEYFFLIGPPLLIIPMYFQYQIIMTMS-----RR---DWVDLAWAISYVM
 201 T.thermophila QHDYKLWQFPFLFKWKLDLSIASYEF-----EGIFLALHWHVLLF

317 M.alpina ATMFLFIKDPVNM--LVYFLVSQAVCGNLLAIVFSLNHNMGMPVISKEEAVDMDFFTKQII
 314 C.elegans G-QLYFLPDWSTR--IMFFLVSHLVCGFLLSHVVTIFNHYSVEKFALSSNIMSNYACLQIM
 298 B.officinalis L-LVSCLPNWGER--IMFVIASLSVTG-MQQVQFSLNHFSSSVYVGK-PKGNNWFEKQTD
 305 M.musculus RFFYTYIPFYGILGALVELNFIREFLESHWFVWVTQMNHLVMEIDLHDH---YRDWFSQLA
 242 T.thermophila N-----QN--FYIVILSELIAGFFSASILVGNHNEMKFER--ITLPEFFEHOIA

375 M.alpina TGRDVH-PGLFANWFTGGLNYQIEHHILFSPMPRHNFSKIQPAVETLCKKYNVRYHTTGM
 371 C.elegans TTRNMR-PGRFIDWLWGGGLNYQIEHHILFPTMPRHNLTVMPLVKEFAAANGLPYMVDDYF
 353 B.officinalis GTLDIS-CPPWMDWFHGGGLQFQIEHHILFPPKMPRCNLRKISPYVIELCKKHNLPPNYASF
 362 M.musculus ATCNVE-QSFFNDWFSCHLNFQIEHHILFPTMPRHNLHKIAPLVKSLCAKHGIEYQEKPLL
 288 T.thermophila ASRNYAFHDIESLLIMGGVQYQTEHHFFFPQIPFYRLPKARVIAEELKKWNLIKIEGPFI



M.alpina	434	EGTAEVFSRLNEVSKAAASKMGKAQ	457
C.elegans	430	TGFWLEIEQFRNIANVAAKLTKKIA	454
B.officinalis	412	KANEMTLRTLRRNTALQARDITKPLPKNLVWEALHTHG	448
M.musculus	421	RALIDIVSSLLKSGELWLDAYLHK	444
T.thermophila	348	EKSHL	352

Figure 5

Structure of delta-6-Desaturase Gene

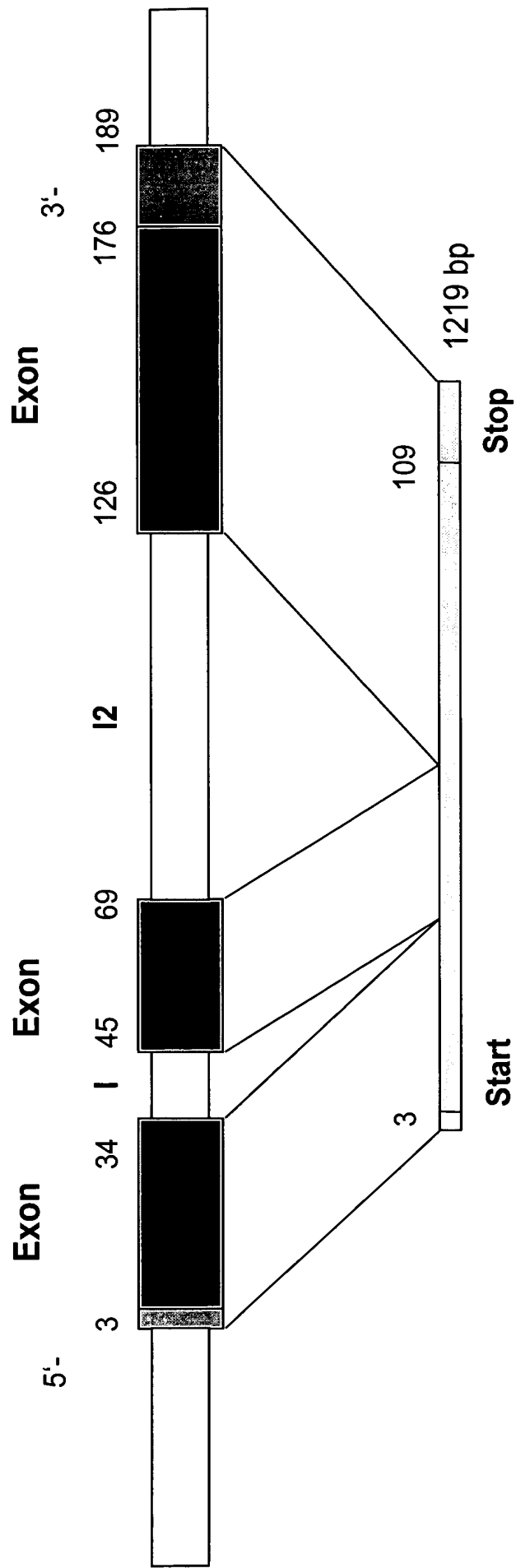


Figure 6

Construction of pBDES6 Expressionconstruct

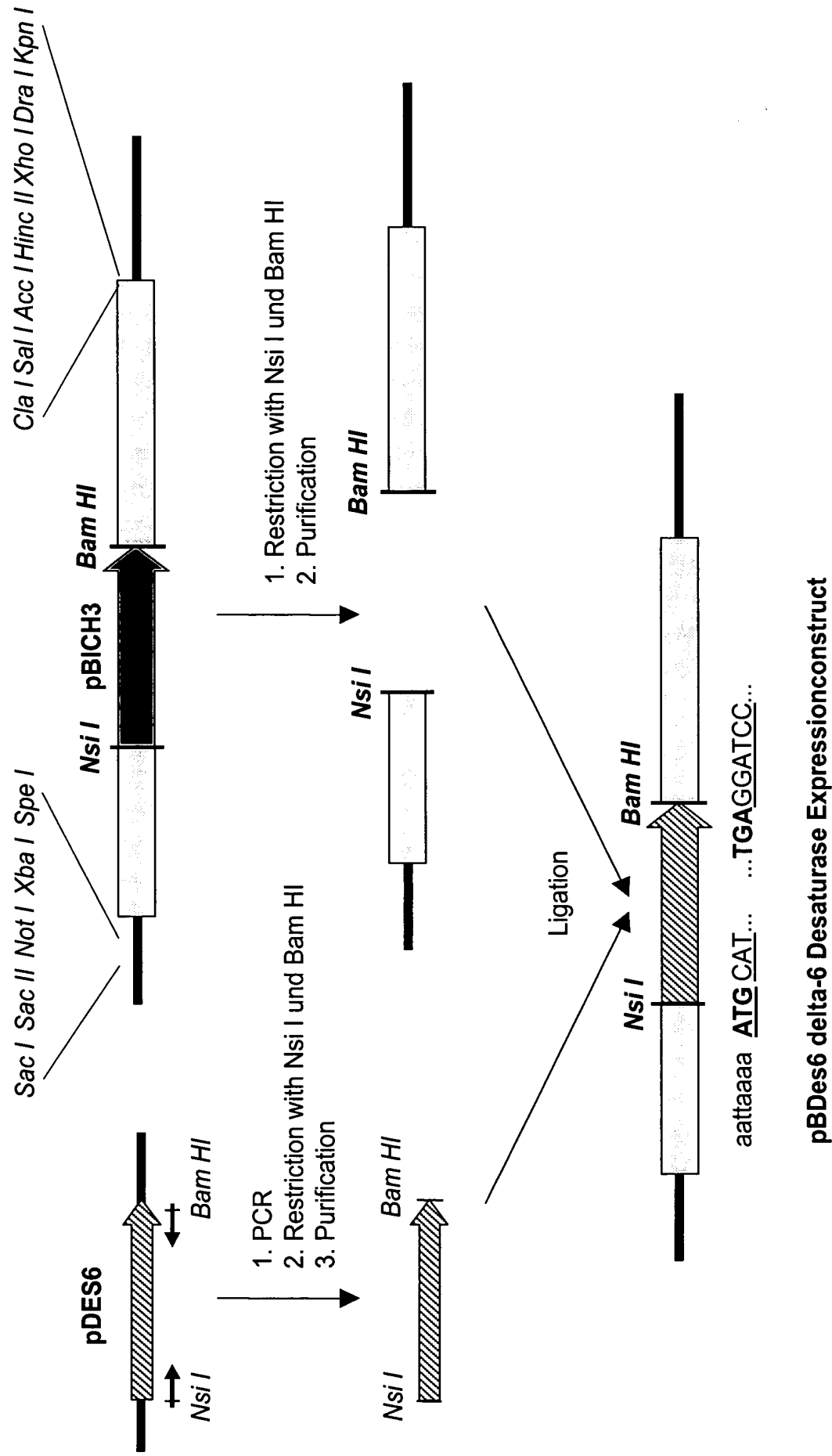


Figure 7

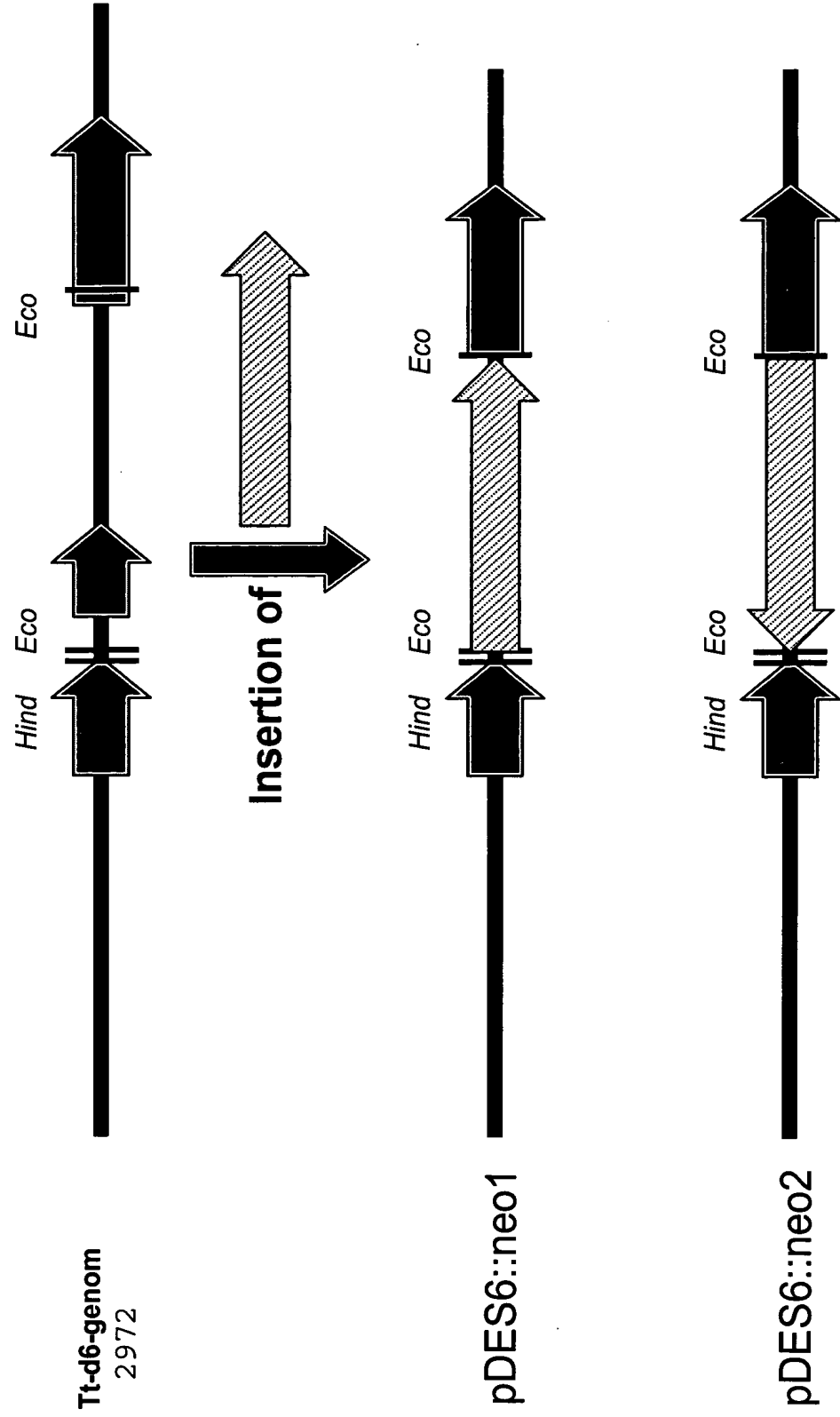
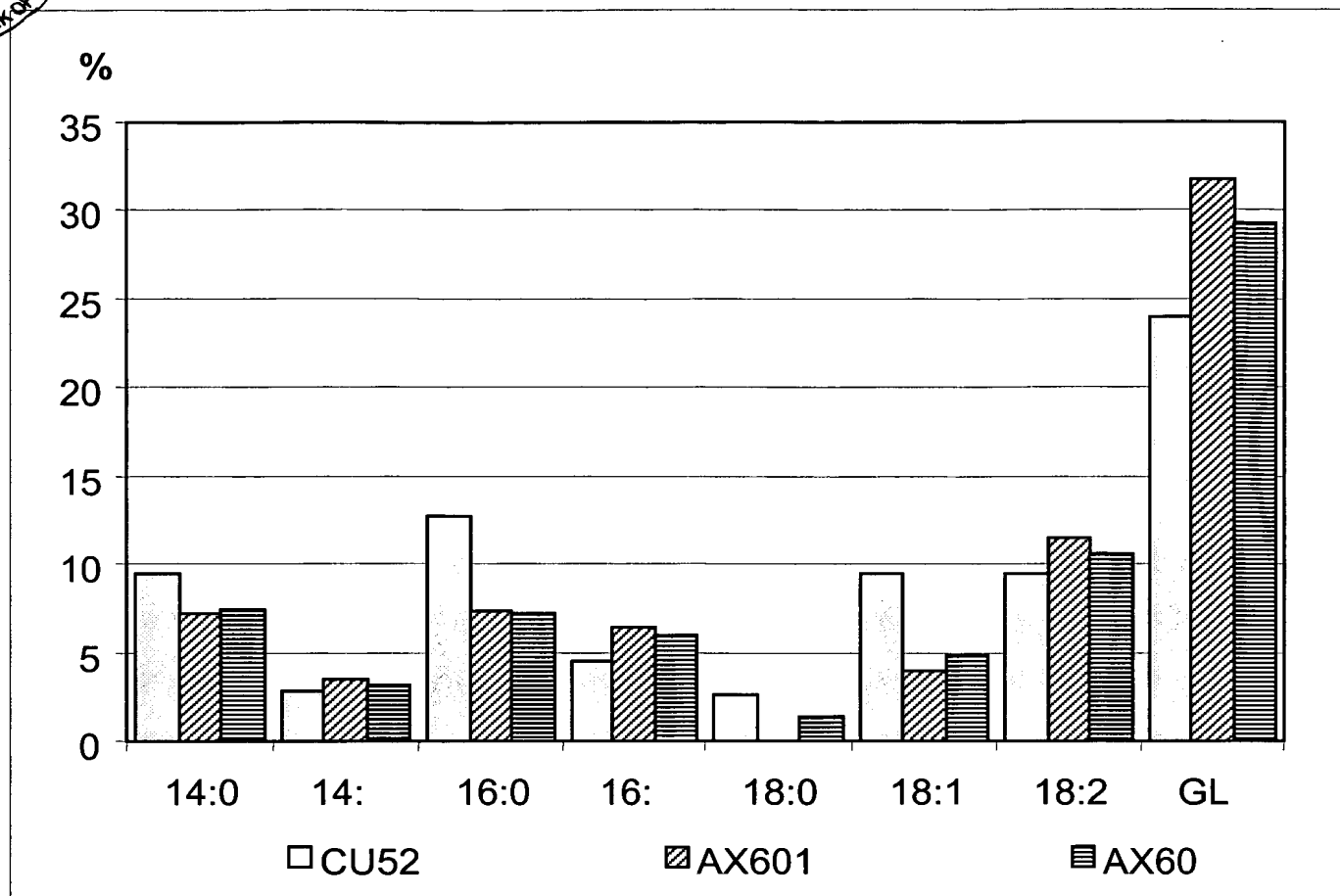


Figure 8



5

Comparison of the fatty acid spectrum (main fatty acids) of the Tetrahymena pBDES6-Transformants (AX601 and AX604) with Tetrahymena wild strain (CU522) after 50 h of cultivation. Value is the percentage of fatty acids relative to total fatty acids.

10